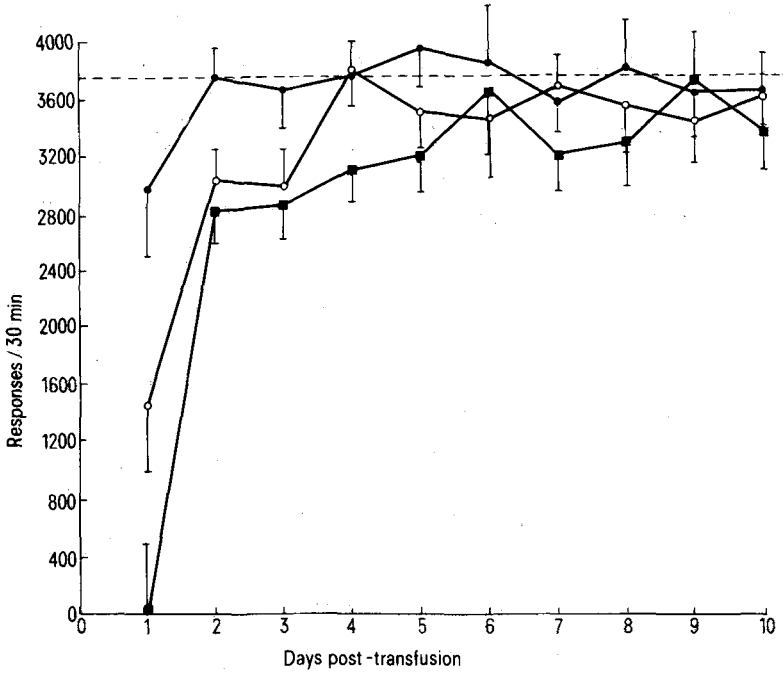

Recovery of fixed ratio operant performance in rats following exchange transfusion with Plasmanate. Each point shows the mean ± SE of 5 rats. The solid circles show the control group. The open circles represent a 50% transfusion and the solid squares a 70% transfusion. Each point has been adjusted for differences in baseline performance. The average baseline response rate for all groups was 3768 ± 84.17.

a decrease in exercise tolerance and work performance in human and animal subjects.

In the present study it was not possible to assess the recovery of the hematocrit or other blood factors due to the possibility of the sampling techniques affecting the behavioral results. However, other studies performed in this laboratory have examined the recovery of blood factors following 70-76% exchange transfusion of rats with albumin[7]. Those studies employed the same surgical and anesthetic procedures as used in this study. The results showed that the hematocrit and oxygen capacity of the blood recovered in about 5-8 days. The course of recovery was similar to the behavioral recovery observed in the present investigation.

1 In conducting the research described in this report, the investigators adhered to the Guide for Laboratory Animal Facilities and Care as promulgated by the Institute of Laboratory Animal Resources, National Academy of Science, National Research Council. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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Intracellular free calcium as a pathogen in cell damage initiated by the immune system[1]

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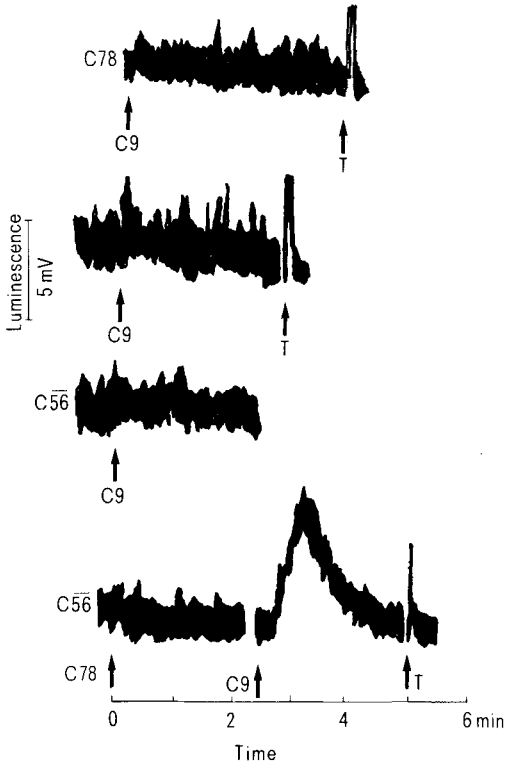
Summary. It is proposed that the earliest intracellular event induced by the action of complement is an increase in cytosolic free calcium, which can occur in the absence of lysis. This increase causes morphological and chemical changes in the cell and also results in modified responses to physiological stimuli.

In many diseases interactions of the immune system occur with tissue antigens involving antibody, complement or cell-mediated responses. Circulating antibodies to constituents of the cell membrane have been identified in several pathological conditions and have effects on the tissues primarily responsible for the clinical manifestations of the disease (table)[3-12]. Although antibodies may interfere with cell function as a direct result of binding to cell surface receptors[4-7], in many cases cell damage is mediated by activation of the complement pathway. A major problem is that it is not yet clear which chemical changes in the cell lead to modifications of tissue function after attack by the immune system.

Using the calcium activated photoprotein oblein trapped in

erythrocyte 'ghosts' as a model system[13-15], we have shown that formation of the terminal complement attack complex[16] causes a rapid increase in intracellular free calcium before release of other ions and macromolecules[13-15]. Much evidence exists that changes in intracellular free calcium concentration mediate the effects of many cell stimuli including the actions of neurotransmitters and hormones on muscle contraction, secretion, cell fertilization, division and intermediary metabolism[17-20]. In addition it has been proposed that intracellular calcium is involved in the action of several drugs including local anaesthetics[21], phenothiazines[22] and cardiovascular agents[23], and in cell injury caused by toxins[24] and anoxia[25]. Abnormalities of structure and function thought to be calcium-dependent have been defined in both red cell and muscle diseases[26-28]. Necrosis[29] and membrane vesiculation[30] have also been shown to be calcium dependent responses of cells to injury.



Complement component C9 increases intracellular free Ca^{2+}. Pigeon erythrocyte 'ghosts', containing the Ca^{2+} activated photoprotein obelin as a marker of intracellular free Ca^{2+} [15,19], were incubated with various functionally pure complement components ($C\overline{56}$ and C78 a kind gift from Professor P.J. Lachmann), extracellular $Ca^{2+} = 1$ mM, estimated intracellular $Ca^{2+} \doteqdot 0.3$ μM[15]. Addition of a purified component C9 (circa 50-80% pure, no detectable other complement components) to $C\overline{5678}$ initiated a rapid increase in intracellular luminescence. It was concluded that C9 binding to the terminal complex $C\overline{5678}$ increases intracellular free Ca^{2+} within a few seconds of binding. This occurs many minutes before cell lysis[13]. Temperature = 37 °C. T = addition of triton (1%, v/v).

In most cells approximately 50% of the calcium is in the nucleus, about 30% in the mitochondria and about 20% in the endoplasmic reticulum. Less than 0.5% of the cell's calcium is in the cytoplasm and only about 0.005% is actually free, the intracellular free calcium being in the range 30-300 nM[19,20]. This means that a relatively small release of calcium from internal stores or an increase in the permeability of the cell membrane to calcium, induced by a physiological stimulus or under pathological circumstances, will cause a large fractional change in cytoplasmic calcium. This can activate or inhibit reactions within the cell, or modify the response of the cell to a physiological stimulus. It has been proposed that such physiological responses are mediated through a group of high affinity calcium binding proteins known as calmodulins[20,31].

Hypothesis. We propose that the earliest intracellular event after formation of the terminal complement attack complex (C5b6789) is an increase in intracellular free calcium. Two levels of free calcium increase are predicted:

1. A rise in cytosolic free calcium within the range 0.1-1 μM will lead to changes in cell structure and function without causing cell lysis. Under these circumstances of mild complement attack, damage to the cell may be reversible, for example, by removal of the terminal attack complex from the cell surface.
2. A rise in cytosolic free calcium to greater than approximately 10 μM in response to more severe complement attack will potentiate membrane damage. This level of free calcium will increase the rate of loss of intracellular constituents leading to lysis and cell death, at which time no free calcium gradient will exist across the plasma membrane.

Evidence in support of the hypothesis. Using a protein, obelin, which emits light when it binds calcium we have measured directly a rapid increase in intracellular free calcium, in pigeon erythrocyte 'ghosts', as a result of the formation of the terminal complement attack complex[13-15]. It was possible to show that after complement activation the intracellular free calcium concentration increased from a resting value of approximately 0.3 μM to 5-30 μM, a concentration at which it remained for at least 1-2 min. The rise in intracellular free calcium occurred before release of other ions and macromolecules, and was dependent on the presence of extracellular calcium. In addition, it was sufficient to explain the inhibition of adrenaline-stimulated adenylate cyclase observed when pigeon erythrocytes or 'ghosts' were incubated with antibody plus complement. A

Diseases where antibodies to plasma membrane antigens have been detected

Disease	Tissue or cell type	Plasma membrane antigen
Haemolytic anaemia[3]	Erythrocyte	Blood group antigens often Rh or I
Myasthenia gravis[4]	Skeletal muscle	Acetylcholine receptor
Graves' disease[5]	Thyroid	Receptor for thyroid-stimulating hormone
Acanthosis nigricans (type B)[6]	Many	Insulin receptor
Allergic rhinitis, Allergic asthma[7]	Lung	β_2 adrenergic receptor
Idiopathic thrombocytopenic purpura[8]	Platelet	Unidentified
Thyroiditis (including Hashimoto's disease)[9]	Thyroid	Unidentified
Pemphigus[10]	Epidermal cells	Unidentified
Chronic active hepatitis[11]	Liver	Unidentified
Diabetes (juvenile onset)[12]	Pancreatic β cells	Unidentified

In several diseases cited antibodies to other cell constituents are also observed.

further consequence of the increased intracellular free calcium concentration was an increased rate of macromolecule release. In separate experiments we have demonstrated a calcium-dependent stimulation of chemiluminescence in phagocytic cells induced by the non-lytic action of anti-cell antibody plus complement[32] and which requires the terminal component C9.

Implications of the hypothesis. Conventionally, attack of a cell by the terminal complement complex (C5b6789) inevitably leads to cell lysis[16]. Yet several complement-mediated morphological and chemical changes have been observed in the absence of lysis in vitro. These include shape changes in erythrocytes[33], reversible membrane depolarization in muscle[34], release of transmitter from frog motor nerve endings[35], inhibition of Schwann cell miniature end plate potentials[36], inhibition of macromolecule synthesis by bacteria[37], release of lysosomal enzyme by cartilage cells[38], release of serotonin by platelets[39] and stimulation of prostaglandin synthesis and bone resorption in an organ culture of foetal rat long bones[40]. In view of the proposed hypothesis it is now necessary to investigate whether these non-lytic effects of complement are mediated by intracellular calcium and, if so, whether they involve the terminal attack complex, C5b6789.

The production of plasma membrane vesicles during complement-mediated lysis[41] and the possible recovery of nucleated cells to complement, mediated through cyclic AMP[42], may also involve interactions with intracellular Ca^{2+}. In addition it has been proposed that the major characteristics of damage by complement are the same as those of cell-mediated damage[43] and that membrane-bound complement components exist on killer lymphocytes[44]. The question now arises whether these types of cell injury are also mediated by an increase in intracellular free calcium.

Terminal complement complexes have been found at motor end-plates of patients with myasthenia gravis[45], and on muscle cells of patients with muscular dystrophy[46]. In muscular dystrophy an increase in nuclear calcium concentration has also been observed[47]. Our hypothesis provides a mechanism by which the immune system may alter cell metabolism and morphology not only in diseases where antibodies to cell surface components have been detected (table) but also in diseases such as rheumatoid arthritis[48], cystic fibrosis[27], muscular dystrophy[45] and multiple sclerosis[49] which may involve other types of interaction with the immune system.

1 In this article the term 'free calcium' is used to mean ionised calcium, i.e. Ca^{2+}, not bound to ligands.

2 Acknowledgments. We are grateful to members of the Welsh National School of Medicine, University of Cambridge, School of Clinical Medicine, and the Marine Biological Association Laboratory, Plymouth, for valuable advice and discussions. In particular we thank our colleagues Dr M.B. Hallett, Dr P.J. Richardson and Mr R.A. Daw. We thank the Science and Medical Research Councils, the British Diabetic Association and the Arthritis and Rheumatism Council for supporting our experimental work.

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